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(54) Enzymatic detergent composition.

(57) The invention relates to a detergent composition comprising lipases. By inclusion of a certain, immunologically defined class of lipases in a detergent composition which comprises a mixture of an anionic and a nonionic detergent, an improved overall detergency is obtained. Typical suitable lipases are obtained from certain Pseudomonas and Chromobacter strains.

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reaction with the Amano-P antibody, using the standard and well-known immunodiffusion procedure according to Ouchterlony (Acta. Med. Scan., 133, pages 76-79 (1950)).

The preparation of the antiserum is carried out as follows:

Equal volumes of 0.1 mg/ml antigen and of Freund's adjuvant (complete or incomplete) are mixed until an emulsion is obtained. Two female rabbits are injected with 2 ml samples of the emulsion according to the following scheme:

day 0 : antigen in complete Freund's adjuvant

day 4 : antigen in complete Freund's adjuvant

day 32 : antigen in incomplete Freund's adjuvant

day 60 : booster of antigen in incomplete Freund's adjuvant

The serum containing the required antibody is prepared by centrifugation of clotted blood, taken on day 67.

The titre of the anti-Amano-P-lipase antiserum is determined by the inspection of precipitation of serial dilutions of antigen and antiserum according to the Ouchterlony procedure. A 25 dilution of antiserum was the dilution that still gave a visible precipitation with an antigen concentration of 0.1 mg/ml.

All lipases showing a positive immunological cross reaction with the Amano-P antibody as hereabove described are lipases according to the present invention. Typical examples thereof are the Amano-P lipase, the lipase ex Pseudomonas fragi FERM P 1339 (available under the trade name Amano-B), lipase ex Pseudomonas nitroreducens var. lipolyticum FERM P 1338 (available under the trade name Amano-CES), lipases ex Chromobacter viscosum, e.g. Chromobacter viscosum var. lipolyticum NRRLB 3673, commercially available from Toyo Jozo Co., Tagata, Japan ; and further Chromobacter viscosum lipases from US Biochemical Corp., U.S.A. and Diosynth Co., The Netherlands, and lipases ex Pseudomonas gladioli.

Preferably, the lipases of the present invention should also show a positive immunological cross reaction with the antibody of one of the the following lipases: lipase ex Chromobacter viscosum var. lipolyticum NRRLB 3673, as sold by Toyo Jozo Co., Tagata, Japan, and lipase ex Pseudomonas gladioli.

Typical examples of such lipases showing such further cross reaction are Amano-P, Amano-B, Amano-CES, lipases ex Chromobacter viscosum, e.g. Chromobacter viscosum var. lipolyticum NRRLB 3673, commercially available from Toyo Jozo Co., Tagata, Japan ; and further Chromobacter viscosum lipases from US Biochemical Corp., U.S.A. and Diosynth Co., The Netherlands, and lipases ex Pseudomonas gladioli.

The lipases of the present invention are included in the detergent composition in such an amount that the final detergent composition has a lipolytic enzyme activity of from 100 to 0.005 LU/mg preferably 25 to 0.05 LU/mg of the composition.

A Lipase Unit (LU) is that amount of lipase which produces 1µmol of titratable fatty acid per minute in a pH stat. under the following conditions: temperature 30°C; pH = 9.0; substrate is an emulsion of 3.3 wt.% of olive oil and 3.3% gum arabic, in the presence of 13 mmol Ca^{2+} and 20 mmol NaCl in 5 mmol Tris-buffer.

Naturally, mixtures of the above lipases can be used. The lipases can be used in their impurified form, or in a purified form, e.g. purified with the aid of well-known adsorption methods, such as a phenylsepharose-packed column technique.

The detergent composition incorporating the lipases of the present invention contains as active detergent material a mixture of one or more nonionic synthetic detergent-active materials and one or more anionic synthetic detergent-active materials. Both types of detergent-active materials are well known in the art, and suitable examples are fully described in Schwartz, Perry and Berch, Surface-Active Agents and Detergents, Vol. I - (1949) and Vol. II (1958) and in Schick, Nonionic Surfactants, Vol. I (1967).

In general, the weight ratio of the nonionic to the anionic detergent varies from 12:1 to 1:12, preferably from 8:1 to 1:8, and particularly preferably from 4:1 to 1:4.

The amount of nonionic and anionic detergent-active material together in the detergent composition ranges from 1 to 30%, usually 2 to 20% and preferably 6 to 16% by weight.

Detergent materials of other types, such as soaps, cationic and zwitterionic detergents, may also be included.

The detergent composition may furthermore include the usual detergent ingredients in the usual amounts. They may be unbuilt or built, and may be of the zero-P type (i.e. not containing phosphorus-containing builders). Thus, the composition may contain from 1-45%, preferably from 5-30% by weight of one or more organic and/or inorganic builders. Typical examples of such builders are the alkali metal ortho-, pyro-and -tripolyphosphates, alkali metal carbonates, either alone or in admixture with calcite, alkali metal citrates, alkali, metal nitrilotriacetates, carboxymethyloxysuccinates, zeolites, polyacetalcarboxylates and so on. Furthermore, it may contain from 1-35% of a bleaching

UV filter in the light pathway and the fatty matter by extracting the dried test cloths with petroleum ether, distilling off the solvent and weighing the resulting fatty matter.

lipase	R^*_{460}	% FM palm oil	% FM olive oil
-	63.9	12.5 ± 0.1	10.0 ± 0.6
Amano-P	70.5	7.2 ± 0.6	6.3 ± 0.6
SP 225	65.0	11.3 ± 0.9	9.8 ± 0.1
Esterase MM	67.3	10.1 ± 0.3	8.7 ± 0.8

These results show that the lipase of the present invention (Amano-P) is superior to the other two prior art lipases.

Example II

Replacing Amano-P by Diosynth as heretofore described in Example I gave similar results.

The following results were obtained:

Example III

The lipase stability of various lipases in a bleach containing detergent composition (5 g/l) containing 3% TAED, 8% sodiumperboratemonohydrate and 0.3% Dequest ® was compared at 30°C in water of 22°GH. The balance of the formulation was equal to the one as described in Example VIII; no Savinase ® or other proteolytic enzyme was present.

Lipase	Residual activity (% of input) 10 min.	30 min.	halftime (min.)
Amano-P	95	99	*
C. viscosum NRRLB 3673	84	73	*
Amano CE (ex Humicola lanuginosa)	100	100	*
Amano AP (ex Aspergillus niger)	83	48	27
Mucor Miehei lipase	61	13	27
Fusarium oxysporum lipase	14	0	3
Esterase MM (ex Mucor mihei)	38	10	7
Lipase PL ex Meito Sangyo, Japan (ex Alcaligenes species)	19	0	3
MY 30.000 ex Meito Sangyo, Japan (ex Candida cylindraceae)	5	0	3

Cotton

	olive oil		palm oil	
	R^*_{460}	%FM	R^*_{460}	%FM
base powder only	67.7	8.8	68.5	9.5
base powder + lipase	75.8	6.2	76.8	5.5
base p. + Savinase + bleach	71.6	8.8	74.3	8.2
base p. + Sav. + bleach + lipase	76.2	7.4	76.2	7.1

These results showed that

-Savinase (bleach) have a large effect on R^*_{460} but no or little effect on %FM

-In contrast to the sensitivity to Savinase in clean detergent solutions shown in Example IV, the lipase is compatible with Savinase/bleach (30GU/ml)/(6/12 TAED/perboratemonohydrate)in these realistic practical wash trials although some inhibition occured.

Example VI

In the same manner as described in Example I, the lipase Amano-P was compared with a lipase producible by Fusarium oxysporum according to EP 0130064. The test cloths were cotton and polyester fabrics, the soiling contained a mixture of palm oil, protein and inorganic pigmentand the water hardness was 8° and 22° GH.

The following results were obtained:

		8° GH		22° GH	
	lipase	R^*_{460}	% FM	R^*_{460}	% FM
cotton	-	60.4	11.2	55.8	15.9
	Amano-P	62.6	8.1	58.7	11.8
	lipase ex Fusarium	63.8	9.9	61.4	13.7
	lipase	R^*_{460}	% FM	R^*_{460}	% FM
polyester	-	67.9	7.4	64.9	8.2
	Amano-P	72.6	4.5	68.1	5.5
	lipase ex Fusarium	70.2	7.3	70.2	7.2

The lipase according to EP 0130064 had a lipolytic activity of 90 LU/mg, but also showed a proteolytic activity of 120 GU/mg. Amano P does not show any detectable proteolytic activity. Although the effects of lipase ex Fusariumon % FM are negligible/small, the effects on R^*_{460} are quite marked. This however, is easily explainable by the proteolytic activity in this lipase sample if a comparison with Example V (powder + Savinase versus powder + lipase) is made.

Example VII

Comparing in the manner as described in Example I the lipase Amano-P with a lipase of the same manufacturer, not according to the invention, Amano CE, and with two otherlipases according to the invention, Amano B and Amano CES gave the following results:

		8° GH		22° GH	
	lipase	R^*_{460}	% FM	R^*_{460}	% FM
cotton	-	73	12.1	70	15.9
	Amano-P	79	6.7	76.5	7.5

		8° GH		22° GH	
	lipase	R^*_{460}	% FM	R^*_{460}	% FM
polyester	-	67.5	9.9	70	10.7
	Amano-P	76.5	8.1	77	9.8

Lipase concentration was 5 LU/ml.

Textile used was cotton.

Example IX

A similar experiment as in Example VIII was done using lipase according to the invention with different resistance against proteolytic enzymes as shown in Example IV.

Lipase	R^*_{460}	% FM
--	67.8	15.5
Amano-P	71.6	11.2
C. viscosum ex Toyo Jozo	74.2	9.5
C. viscosum ex Diosynth	72.9	10.3

Residual activities in the wash liquor after the 30 minutes wash process:

Amano-P 36%

Toyo Jozo 55%

Diosynth 60%

Detailed comparison with Example IV shows that in the realistic, practical wash conditions used in this Example lipases of the invention are substantially less sensitive to attack by proteases such as Savinase used in detergent products.

Example X

The test of Example 1 was repeated, but using 4 g/l of the detergent composition and using lipases in an amount of 1 LU/ml. The following results were obtained:

0		73.7	67.8	10.6	9.0
1 LU Toyo Jozo		78.8	72.7	6.9	5.1
3 LU Toyo Jozo		79.7	73.7	7.1	4.7
1 LU lipase ex	Pseudomonas gladioli	79.9	73.3	6.6	4.9
3 LU lipase ex	Pseudomonas gladioli	80.7	74.7	7.3	4.6

Example XII

Repeating Example I, using the detergent composition of Example I at 4 g/l in water of 8° GH, or the detergent composition of Example VIII at 5 g/l in water of 22° GH, at various temperatures gave the following results:

Composition	Toyo Jozo lipase (LU/ml)	Temperature (°C)	R*460 palm oil	R*460 olive oil	% FM palm oil	% FM olive oil
"	0	30	64.3	61.4	14.5	16.0
"	3	30	74.2	72.6	7.4	7.6
"	0	40	68.2	64.8	12.5	13.7
"	3	40	75.9	74.2	6.5	6.9
"	0	50	68.9	68.3	12.3	11.8
"	3	50	76.4	75.1	6.1	6.4
Composition of Example VIII	0	30	73.9	74.7	8.4	7.9
"	3	30	75.4	76.1	7.6	7.0
"	0	40	74.8	75.0	7.5	7.8
"	3	40	76.1	76.3	6.9	7.1
"	0	50	75.3	75.4	7.5	7.7
"	3	50	76.9	76.8	6.1	7.6

Example XIII

In the manner as described in Example I, the following detergent compositions were tested.

A: 9 % anionic detergent

1 % nonionic detergent

21.5 % sodium tripolyphosphate

7 % sodium perborate

0.6 % Savinase (a proteolytic enzyme)

balance sodium sulphate + minor ingredients

B: 9 % anionic detergent

4 % nonionic detergent

28% zeolite

Claims

1. A detergent composition comprising a mixture of an anionic and a nonionic detergent-active compound and an enzyme, characterised in that the enzyme is a lipase which shows a positive immunological cross-reaction with the antibody of the lipase, producible by the microorganism Pseudomonas fluorescens IAM 1057.

2. A composition according to claim 1,

characterised in that the lipase also shows a positive immunological cross-reaction with the antibody of the lipase, producible by the microorganism Chromobacter viscosum var. lipolyticum NRRLB 3673 or Pseudomonas gladioli .

3. A composition according to claim 1 or 2,

characterised in that the positive immunological cross-reaction showing lipase is a lipase, producible by strains of the Pseudomonas and the Chromobacter genus.

4. A composition according to claim 3,

characterised in that the lipase is producible by strains of Pseudomonas fluorescens, of Pseudomonas fragi, of Pseudomonas nitroreducens var. lipolyticum, of Pseudomonas gladioli, and of Chromobacter viscosum.

5. A composition according to claims 1-4,

characterised in that the composition contains the lipase in such an amount, that the final composition has a lipolytic enzyme activity of from 0.005 to 100 LU (lipase units) per mg.

6. A composition according to claims 1-5,

characterised in that it further contains a bleaching agent.

7. A composition according to claims 1-7,

characterised in that it further contains a proteolytic enzyme.

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EUROPEAN SEARCH REPORT

Application Number

EP 86 20 0941

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	DE-A-1 932 981 (TOYO JOZO K.K.) * Page 10, lines 11-14; claims 1,3 *	1,3,4	C 11 D 3/386 C 12 N 9/20
A	FR-A-2 362 399 (EASTMAN KODAK) * Page 10; claims 1,5,7-11 *	1,3	
D,A	FR-A-2 121 170 (UNILEVER) * Example 4; claims 1-6,8 *	1	
A	DE-A-2 061 033 (HENKEL) * Examples; claims *	1,6,7	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

C 11 D
C 12 N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	25-08-1988	PFANNENSTEIN H.F.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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